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PROJECT TITLE:	BIOLOGICAL EFFECTS OF SMOKE
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PROJECT LEADER:	R. A. Pages
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1. YEAST MITOTIC GENE CONVERSION ASSAY[1]

A. Whole Smoke (WS) and Gas Phase (GP) Activity (with 6908)

The observation that burley WS collected in DMSO was more active than conventional dry impaction trapped (IT) burley WSC was confirmed and extended in the experiments conducted this month. When GP from burley cigarettes was recombined with dry IT WSC, the activity of the recombined sample was similar to that of burley WS collected in DMSO. [GP from burley cigarettes was previously shown to be inactive in this system.[2]]

The dry IT WSC, GP, and WS as well as a recombined sample were obtained from a low tar filler cigarette (LTF-IIA). When tested, the activities were similar to that obtained with the burley cigarette. That is, WS > recombined sample > dry IT WSC. Unlike the GP from burley, that of the low tar filler cigarette was active. These results are encouraging because they show that different collection procedures are trapping different and/or more active agents.

B. Cell Stock Sensitivity

Recent experiments involving another flask of the same batch of cells we had been using in this assay showed this new flask of cells to be extremely sensitive (more active) to WSCs. The spontaneous conversion frequency and the response to a pure chemical were similar regardless of which flask of cells was used. Only the response to WSCs were different, however, the relationship between two WSCs remained the same (*e. g.*, IM > 2R1). If we could determine what factor(s) is responsible for the increased sensitivity of the cells in this flask, we would have made an important contribution to understanding how this assay works. One problem experienced by ourselves and others[3] is the low response of most agents when tested in this system.

2. *SALMONELLA*/MICROSOME ASSAY

A. Pyrolysis (with 6908)[4]

As reported previously,[5] the pyrolyzate from 2R1 filler pyrolyzed at 200 and 300°C was not active. These studies were extended at pyrolysis temperatures of 350, 400, and 500°C. Increasing activities (TA98 with microsomes) were observed as the temperature was increased.

B. Optimizing the Activity of Highly Active Fractions[4]

Others have reported that the response of very active samples is reduced as the microsomal concentration is increased.[6] To determine if the standard microsome concentration (35 µl/plate) employed in our laboratories was not yielding misleading results, three different volumes of microsomal mix were tested with a series of HPLC or LC purified burley WSC fractions. As expected, the lowest level of microsomes tested (15 µl/plate) produced the highest specific activities. However, the relationship of one fraction to another remained unchanged regardless of the microsomal concentration used. Thus, the active fraction(s) will probably not be missed using the standard level of microsomes.

C. Assay Modifications[7]

In an attempt to streamline the assay protocol, several experiments have been done to examine the effects of freshly dosing *versus* predosing the *Salmonella* cells. Also, we have examined the question of culture preparation as it pertains to occasionally high spontaneous backgrounds. While no firm conclusions can be made at this time (more experiments are needed), the results are similar regardless of the method used to dose the cells. The question of occasional high spontaneous backgrounds is still unanswered but does not appear to be related to how the culture was prepared from the master plates.

D. Additive and WSC Testing[7]

Three samples were tested at the request of J. L. Charles and a memo was issued concerning the results.[8]

3. L5178Y THYMIDINE KINASE MUTATION ASSAY

A. A Pure Chemical Negative Control Compound[9,10,11]

Efforts are still in progress to find a negative control compound for this system. Everything tested thus far (succinic anhydride, nicotine, ethanol, and DMSO) that we thought would be negative has been positive. Recently, methanol and anthracene have been examined. Results on the former compound are not complete while those concerning the latter agent suggest it is negative. However, the anthracene data were clouded by solubility problems with the stock solution. In order to obtain significant cell killing, a precipitate was observed in the stock solution as well as in the treated culture.

Our studies with ethanol have now been completed and as indicated above ethanol is weakly positive although other investigators have reported it to be negative.[12] It was interesting that when first

examined, ethanol was negative[13] while more recent experiments have shown it to be positive. This discrepancy was resolved when it was found that two different microsomal preparations were used in the experiments just described. In work just completed, it was conclusively shown that one microsomal prep was capable of activating ethanol while the other was not. While the *in vivo* activity of ethanol is questionable, the *in vitro* studies suggest that its primary metabolite (acetaldehyde) is responsible for its activity. Studies are underway to determine if acetaldehyde is active in this assay. If successful, these studies would complement those done in *E. coli* concerning the activity of aldehydes in that system.[14]

4. BHK CELL TRANSFORMATION[15]

A collaborative study with Dr. J. A. Styles of ICI in England was completed this month. A cell clone isolated in our laboratories yielded a high spontaneous transformation frequency when tested in the ICI protocol using their media, serum *etc.* Using ICI's cells and their protocol with several different types of sera produced some differences and some similarities depending on what parameter was measured. For example, the cloning efficiencies and transformation frequencies were similar while the LD_{50}s were quite different. The type of sera used contributes to the variation observed in this assay. We are attempting to obtain a contaminant-free flask of ICI's cells, so that more studies can be done with this system.

5. REFERENCES

1. Thompson, L. H. Notebook No. 7557, pp. 7-12.
2. Pages, R. A. 6906 Monthly progress report. Monthly Progress Reports 80-180. 1980 July 15.
3. Thompson, L. H.; McCuen, R. W. Trip Report: The 11th annual meeting of the Environmental Mutagen Society (EMS), Nashville, Tennessee, March 16-19, 1980. Memo to R. A. Pages. 1980 April 1.
4. Drew, S.; McCoy, W. R. Notebook No. 7484, pp. 105-108.
5. Ferguson, R. N. 6908 Monthly progress report. Monthly Progress Reports 80-180. 1980 July 15.
6. Ishii, K.; Yamazoe, Y.; Kamataki, T.; Kato, R. Metabolic activation of mutagenic tryptophan pyrolysis products by rat liver microsomes. Cancer Research. 40:2596-2600. 1980 July.

5. REFERENCES *(continued)*

7. Rapp, K. E.; Tickle, M. H. Notebook No. 7437, pp. 5-8.
8. Rapp, K. E.; Tickle, M. H. F80-8, -9, and -10: *Salmonella*/microsome activity. Memo to R. A. Pages. 1980 July 25.
9. Meyers, R. Notebook No. 7465, p. 132.
10. McCuen, R. Notebook No. 7508, p. 65.
11. Ayers, D.; Penn, J. M. Notebook No. 7551, pp. 17, 18.
12. Amacher, D. E.; Paillet, S. C.; Salsburg, D. S.; Roy, V. A. Analyses of mouse lymphoma TK assay results for statistical significance via a two sample t test. 10th Annual Meeting of the Environmental Mutagen Society, New Orleans; March 8-12, 1979; p. 82.
13. Penn, J. M. Notebook No. 7378, pp. 149-150.
14. Warfield, A. H. Determination of carbonyl compounds in cigarette smoke by reversed-phase high performance liquid chromatography. Special report 80-130. 1980 May 12.
15. Weissbecker, L. Notebook No. 7467, pp. 79-81.

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